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THE STUDY OF MONOAMINE OXIDASE ACTIVITY BY HISTOCHEMICAL PROCEDURES

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Abstract—Histochemistry gives more information of the nature of the activity of monoamine oxidase than can be obtained readily by conventional biochemical procedures. Thus in sections of human endometrium which have been frozen and sectioned by a special technique, this enzyme has been shown to occur either in discrete particles or diffusely, depending on the stage of the menstrual cycle. The physiological significance of the changed localization of this enzyme might be related to the spasm of the spiral arteries which is said to initiate menstruation.

STUDIES on monoamine oxidase have indicated that striking changes in the activity and distribution of this enzyme may occur as a consequence of an increased local concentration of histamine (Diengdoh, unpublished data). It seemed, therefore, that direct histochemical observation on this enzyme might be of value. Such attempts as have been made, notably by Yasuda and Montagna[1] (also in Burstone[2]), are open to objection on two grounds; firstly, that the preparation of the tissue was unsuitable for the degree of preservation of cellular structure that is required for accurate histochemistry, and secondly that the sections were so thick that cellular detail was rendered too indistinct to allow the activity to be determined accurately within the cells.

The development of a controlled temperature freeze-sectioning technique[3] made it feasible to overcome these difficulties. By this procedure, unfixed tissue can be sectioned at 8μ; not only are mitochondria preserved in such slices but the permeability of the lysosomal membrane appears to be unaffected.[4, 5] When a histochemical procedure for demonstrating monoamine oxidase was applied to such unfixed sections of various tissues, this activity was found variously in cytoplasmic particles, of the size of mitochondria, or diffusely in the cytoplasm. There was some indication, however, that this change of localization might have been induced by physiological variations. Thus it was possible that a suitable test material might be the human endometrium since this tissue shows striking changes induced by altered physiological states; moreover there is some indication[6] that the onset of menstruation may be related to spasm of the spiral arteries and hence to the detoxication of catechol amines. Consequently a study was made of the histochemical activity and localization of monoamine oxidase in biopsies of human endometrium, taken at different stages of the menstrual cycle.

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MATERIALS AND METHODS

Biopsies of human endomentrium were obtained by curettage. They were immediately frozen against the side of a pre-cooled tube which had been equilibriated to the temperature of solid carbon dioxide. The tissue was cut at about 8μ in a cryostat microtome, refrigerated to $-25°$, as described by Cunningham *et al.*[3]

The sections were caused to adhere to a warm dry slide, and left in the refrigerated cabinet until required; this was never longer than about one hour. A drop of the warm incubation medium was placed on the section, which was then left for two hours in a humidity chamber at 37°. They were rinsed in distilled water and mounted in Farrants' medium.

The incubation medium differed from that suggested by Pearse[7] only in that no sodium sulphate was added: it therefore consisted of tryptamine hydrochloride (25 mg.), Nitroblue tetrazolium (5 mg.), 0·1 M phosphate buffer at pH 7·6 (5 ml.), distilled water (15 ml.).

To provide some control of this reaction, serial sections were incubated similarly in a medium from which the tryptamine had been excluded.

RESULTS

At all stages of the menstrual cycle, except close to the menstrual period itself, the enzyme reaction occurred in discrete particles. In the proliferative phase, in particular, the only staining with the formazan was found in small particles which were more noticeable in the glands than in the stroma (Fig. 1A). Although the distribution of the active stromal cells seemed to be rather random, it was clearly not related to that of the blood vessels. Towards the end of the proliferative phase some diffuse colour was also seen particularly at the lumenal margin of the glandular cells. The particles in the region of the diffuse colour also appeared to be swollen (Fig. 1B). As the secretory phase progressed, a greater proportion of the granules were large and swollen; the diffuse colour became progressively accentuated throughout cells of the glands, with a concomitant loss of granules from the lumenal margin (Fig. 1C). Finally the glandular cells, having shown progressive loss of the particles, reacted to produce intense diffuse staining which outlined the unstained nuclei, while the cells of the stroma, closest to the glands, showed swollen stained particles (Fig. 1D). To a less marked extent, this pattern of staining was seen also in certain other active areas of the stroma, not related to the blood vessels.

DISCUSSION

It has been possible to demonstrate the exact location of amine oxidase activity because a hydrogen-acceptor of relatively high electrode potential and high substantivity for protein[8, 7] has been used in a histochemical test on relatively thin sections of unfixed endometrium in which cellular detail is well preserved.[9] If the oxidative deamination process were simple, i.e.

$$R - CH_2.\overset{+}{N}HR_1 + H_2O + O_2 \rightarrow R.CHO + \overset{+}{N}H_2R_1 + H_2O_2 \qquad 1.$$

then it would be difficult to see how it could reduce the tetrazolium salt. Blaschko,[10] as a consequence of comparing manometric results with those obtained by using

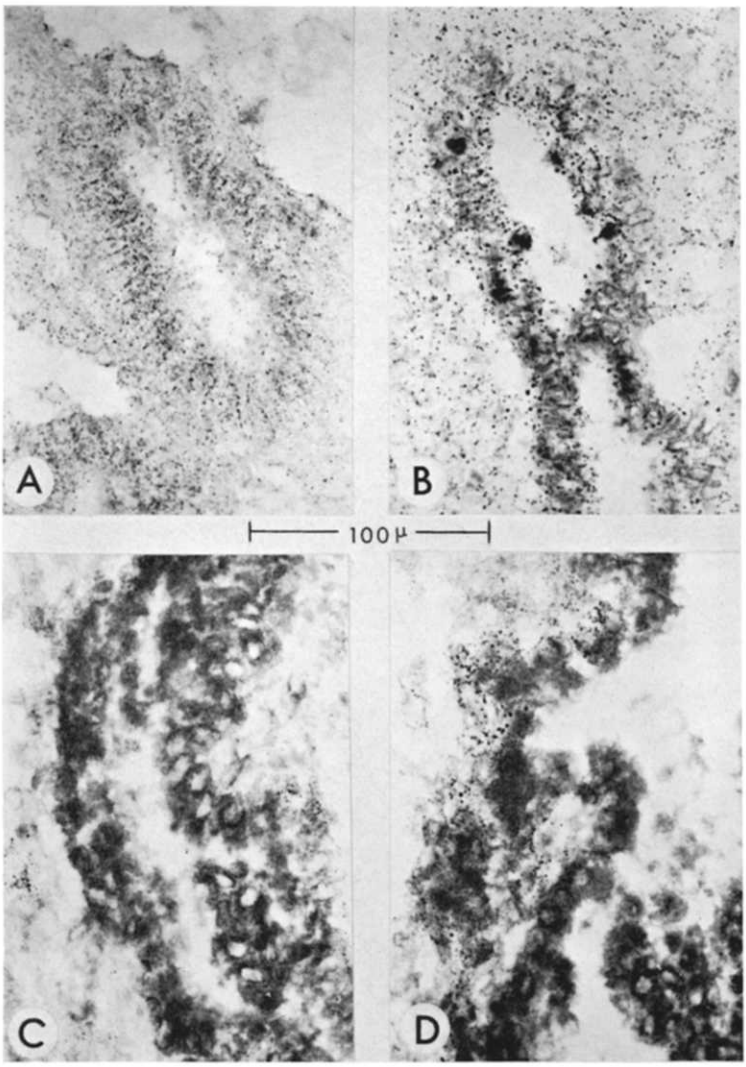

FIG. 1. Amine oxidase activity in controlled-temperature frozen sections of endometrium.

(A) In the early proliferative phase, the reaction, which is predominantly in the glands, is confined to discrete particles.

(B) Later in the proliferative phase, there is diffuse staining of the glands. There are fewer particles in these cells and many appear swollen.

(C) In the secretory phase, the diffuse staining of the glands is more intense and outlines the nuclei; particles are present only in the stromal cells.

(D) Towards the end of the secretory phase, the particles in the stromal cells adjacent to the glands appear swollen. The glands still show intense diffuse staining.

triphenyltetrazolium chloride under anaerobic conditions, was led to suggest that, in the living organism, the enzyme may not react directly with molecular oxygen but rather with another, as yet unidentified, hydrogen acceptor. In this case, the histochemical system might be of the following form (see also[11, 12]):

$$R - CH_2.\overset{+}{N}HR_1 + A \xrightarrow[\text{monoamine oxidase}]{-2H} R - CH{:}\,\overset{+}{N}R_1 + AH_2$$

where A is the hydrogen acceptor which can reduce nitroblue tetrazolium

$$R - CH{:}\,\overset{+}{N}R_1 + H_2O \rightarrow R - CHO + \overset{+}{N}H_2R_1$$

$$AH_2 + O_2 \rightarrow A + H_2O_2$$

Biochemical studies have shown that monoamine oxidase is associated with particles which have sedimentation properties akin to those of mitochondria.[12] In the endometrium, during the proliferative phase, the histochemical test showed that this activity was located almost exclusively in small particles of the size of mitochondria. However, the histochemical evidence does not prove that the enzyme is situated in mitochondria because in the cells of this tissue it has proved impossible to observe structures which correspond to the conventional cytological definition of mitochondria (unpublished data).

The changes in localization of monoamine oxidase activity with the menstrual cycle, from small particles with almost no diffuse colour, to swollen particles with more diffuse activity, culminating in the apparent loss of particles but accentuation of the diffuse colour, make it likely that the particulate localization of the enzyme reflects its true location. Moreover, from these results, and from those of the effect of histamine on such particles (Kirkby and Chayen in preparation), it seems possible that the physiological function of monoamine oxidase may be affected by its situation within the cell. Such an effect might occur if its activity were to be dependent on its close apposition either to a hydrogen-accepting system as suggested by Richter[11] and by Blaschko,[10] or to a peroxidase, for the removal of peroxide formed as in Equation 1. Any loss in physiological activity which might be expected to arise from disturbance to the cytoplasmic particles, would alter the effective level of catechol amines in the uterus and so induce spasm of the spiral arteries which initiates menstruation.

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